Institute Report No. 294

Mutagenic Potential of JA-2 Solid Propellant in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

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DIVISION OF TOXICOLOGY

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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Mutagenic Potential of JA-2 Solid Propellant in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 149)--Sano and Korte

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for Richard A. Kishimoto 28 Sept 88

Edwin S. Beatrice (date)
COL, MC
Commanding

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The mutagenic potential of JA-2 solid propellant was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, and TA102 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.

Keywords: JA-2 solid propellant; Ames Salmonella/Mammalian Microsome Mutagenicity Test; [illegible]

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ABSTRACT

The mutagenic potential of JA-2 solid propellant was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, and TA102 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, JA-2, DEGDN, Solid Propellant

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

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US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5012
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: #3E162720A835/180/TLB0

GLP STUDY NUMBER: 85014

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: SGT Steven K. Sano, BA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired stability and purity data on the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: JA-2 Solid Propellant

INCLUSIVE STUDY DATES: 19 Aug - 30 Aug 85

OBJECTIVE: The objective of this study was to determine the mutagenic potential of JA-2 solid propellant (LAIR Code TP056) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

CPT John W. Harbell, PhD, MSC; SGT Lillie D. Witcher, BS; SP4 John R.G. Ryabik, BS; Mr. John Dacey; and Ms. Joanne Wong provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP study number 85014 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte Jr 14 July 88
DON W. KORTE JR, PhD / DATE
MAJ, MSC
Study Director

Conrad Wheeler 14 July 88
CONRAD WHEELER, PhD/ DATE
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DEPARTMENT OF THE ARMY

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REPLY TO
ATTENTION OF:

SGRD-ULZ-QA (70-1n) 15 September 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85014

1. This is to certify that in relation to LAIR GLP Study 85014, the following inspections were made:

16 August 1985	-	Protocol Review
27 August 1985	-	Plate Incorporation

2. The institute report entitled "Mutagenic Potential of JA-2 Solid Propellant in the Ames Salmonella/Mammalian Microsome Mutagenicity Test,"Toxicology Series 149, was audited on 20 July 1988.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

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Mutagenic Potential of JA-2 Solid Propellant in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test--Sano and Korte

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Division of Toxicology, LAIR, to conduct an initial evaluation of the health effects of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity test, acute oral toxicity tests in rats and mice, acute dermal toxicity tests in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs. This report contains the results of a study that assessed the mutagenic potential of JA-2 in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (2).

This evaluation of JA-2 utilizes a revision of the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (3). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set. TA97 replaces TA1537, TA1535 and TA1538 which are removed from the recommended set. TA98 and TA100 are retained.

Objective of the Study

The objective of this study was to determine the mutagenic potential of JA-2 solid propellant (LAIR Code TP056) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Compound name: JA-2 Solid Propellant

Code number: LAIR Code No. TP056

Physical state: Solid

Source: Hercules Incorporated
Wilmington, Delaware

Storage: JA-2 was received from Radford Army Ammunition Plant (Radford, VA) and assigned the LAIR Code number TP056. The test compound was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Data provided by Hercules Inc., characterizing the chemical composition and purity of the test material, are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals and the test compound were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO).

Chemical Preparation

JA-2 was stored at room temperature (21°C) until used. The solid propellant was ground into a fine powder with a liquid nitrogen freezer/mill Model # 6700 (Spex Industries). On the day of dosing, 300 mg of the test compound was measured into a sterile vial and dissolved in 6 ml of grade I dimethyl sulfoxide to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA97, TA98, TA100, and TA102, obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (4).

Test Format

JA-2 was evaluated for mutagenic potential according to a revised Ames method (3). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (4).

Toxicity Tests

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of JA-2 ranging from 1.6 x 10^{-3} mg/plate to 5 mg/plate, and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed a decrease in the number of macrocolonies (below the number in the spontaneous reversion plates) or an observable reduction in the density of the background lawn, a maximum "limit" dose of 5 mg/plate was used in the mutagenicity test.

Mutagenicity Test

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Microbiological Associates Inc. (Bethesda, MD). The optimal titer of this S-9, as determined by Microbiological Associates Inc., was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (5). The water used in this medium and in all reagents came from a Technic Model 301 Reverse Osmosis Pre-Treatment Water System (Seattle, WA), LAIR SOP, OP-STX-94 (6). Plates were

incubated upside down in the dark at 37°C for 72 hr. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Maron and Ames (3). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The *Salmonella* strains were verified by a standard battery of tests. The integrity of the different *Salmonella* strains used in the assay was verified by the following standard tests:

-Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer of the cell wall is present.

-Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor.

-Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism (for all strains except TA102).

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene, 2-aminofluorene, 2-aminoanthracene and 4-nitroquinoline-n-oxide, were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (7), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (3) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations/Changes

A 72-hr rather than a 48-hr incubation period was used. According to Maron (personal communication, 1985), the additional 24-hr growth enables all of the revertant colonies, especially TA102, to be detected with the colony counter.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

On 23 August 1985, the toxicity of JA-2 was determined (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 1). A 33% reduction in number of revertants was observed after exposure of the tester strain (TA100) to the 1 and 5 mg/plate concentrations of JA-2. Since the background lawn was normal and these values fell within the range historically observed for negative control runs, a high dose of 5 mg/plate was selected for the mutagenicity assay.

Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 27-30 August 1985 (Table 2). JA-2 did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3). However, tester strains TA97 and TA98 exhibited toxicity as

TABLE 1: TOXICITY DETERMINATION FOR JA-2

GLP STUDY NUMBER 85014 23 Aug 1985 PERFORMED BY SANO/WONG

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

CONCENTRATION OF TEST COMPOUND	MEAN	(1SD)	BACKGROUND LAWN*
START RUN NEGATIVE CONTROL	102	(14.0)	NL
5.0 mg/plate	68	(4.7)	NL
1.0 mg/plate	68	(7.0)	NL
0.2 mg/plate	77	(4.2)	NL
0.04 mg/plate	92	(8.5)	NL
0.008 mg/plate	88	(7.6)	NL
0.0016 mg/plate	87	(0.6)	NL
END RUN NEGATIVE CONTROL	96	(8.1)	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION (TA100)

HISTIDINE REQUIREMENT	NG*	
AMPICILLIN RESISTANCE	G	
UV	NG	
CRYSTAL VIOLET SENSITIVITY (ZONE SIZE)	NG	(12mm)
STERILITY CONTROL	NG	

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

* NL = Normal Lawn G = Growth NG = No Growth

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF JA-2 (TP056)

GLP STUDY NUMBER 85014 12 SEP 1985 PERFORMED BY SANO/WONG

STRAIN VERIFICATION

STRAINS	OBSERVATIONS* TA97	TA98	TA 100	TA102
HISTIDINE REQUIREMENT	NG	NG	NG	NG
AMPICILLIN RESISTANCE	G	G	G	G
UV REPAIR	NG	NG	NG	G
CRYSTAL VIOLET SENSITIVITY (ZONE SIZE)	NG (13mm)	NG (10mm)	NG (9mm)	NG (10mm)
STERILITY CONTROL	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

* NL = Normal Lawn G = Growth NG = No Growth

TABLE 3: MUTAGENICITY ASSAY FOR JA-2 (TP056)

STUDY NUMBER: 85014 DATE: 12 SEPT 85 PERFORMED BY SANO/WONG

COMPOUND	DOSE	TA97	TA98	TA100	TA102
		WITHOUT S-9			
NEG CONTROL	0.0 mg/ml	57 (7.2)†	27 (4.4)	108 (12.0)	185 (6.2)
NQNO*	2.0 µg/ml			483 (335.1)	690 (53.3)
TP056	5.0 mg/plate	28 (8.7)	20 (4.6)	103 (7.6)	181 (28.6)
TP056	1.0 mg/plate	68 (11.2)	25 (1.5)	94 (8.0)	207 (27.3)
TP056	0.2 mg/plate	83 (2.6)	26 (6.4)	95 (11.1)	205 (10.1)
TP056	0.04 mg/plate	66 (15.4)	24 (3.2)	110 (10.8)	197 (22.8)
TP056	0.008 mg/plate	51 (6.1)	26 (1.5)	91 (1.5)	179 (18.5)
TP056	0.0016 mg/plate	52 (18.7)	23 (4.2)	94 (13.2)	158 (10.4)
		WITH S-9			
NEG CONTROL	0.0 mg/ml	74 (18.0)	34 (4.6)	94 (11.6)	262 (10.7)
AF*	2.0 µg/ml	352 (22.1)	1157 (168.2)	547 (14.6)	387 (27.3)
BP*	2.0 µg/ml		528 (26.7)	471 (30.5)	
AA*	2.0 µg/ml		1195 (275.1)	1132 (72.2)	
TP056	5.0 mg/plate	16 (3.5)	13 (2.3)	88 (6.7)	189 (53.5)
TP056	1.0 mg/plate	81 (4.7)	23 (3.6)	96 (9.0)	218 (27.5)
TP056	0.2 mg/plate	75 (9.3)	36 (5.6)	87 (5.9)	210 (65.6)
TP056	0.04 mg/plate	77 (12.7)	33 (6.5)	79 (14.6)	250 (3.2)
TP056	0.008 mg/plate	61 (8.0)	27 (4.4)	77 (2.6)	252 (22.5)
TP056	0.0016 mg/plate	60 (2.1)	29 (1.5)	77 (5.5)	238 (17.3)

† Values represent the mean number of revertants/plate (± standard deviation)
* NQNO = 4-nitroquinoline-n-oxide, AF = 2-aminofluorene, BP = benzo[a]pyrene, AA = 2-aminoanthracene

indicated by a reduction in revertant counts at the 5 mg/plate concentration.

A copy of the raw data is included in Appendix B.

DISCUSSION

Certain test criteria must be satisfied before an Ames test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of the Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, JA-2 was evaluated in the Ames test. Criteria for a positive response are a correlated dose-response relationship and a twofold increase in revertant colony counts relative to the respective negative control counts (3,4,7). JA-2 did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that JA-2 is not mutagenic when evaluated in the Ames test.

CONCLUSION

JA-2 was evaluated for mutagenic potential in the Ames Test, both in the presence and absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.

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6. Operation of the Technic Model 301 Reverse Osmosis Pre-Treatment Water System and the Corning Model MP-1 Glass Still. LAIR Standard Operating Procedure OP-STX-94, Presidio of San Francisco, California: Letterman Army Institute of Research, 29 July 1985.

7. Brusick D. Genetic toxicology. In: Hayes AW, ed. Principles and methods of toxicology. New York: Raven Press, 1982:223-272.

Appendix A: CHEMICAL DATA

Test substance: JA-2 Solid Propellant

Composition/Analytical data: See appended data sheet for information supplied by source.

LAIR Code No: TP056

Physical state: solid black cylinders

Preparation of test substance for dosing:

The cylinders of JA-2 were ground to a fine powder under liquid nitrogen using a Spex freezer mill. After sieving the powder through an 80-mesh screen, the JA-2 was dissolved in DMSO.

Source: Radford Army Ammunition Plant, Radford, Virginia (prime contractor: Hercules Inc, Wilmington, Delaware)

Lot No.: RAD83K001S153

Appendix A (cont.): CHEMICAL DATA

DATA SHEET

Ingredient	FORMULA JA-2 Finished Propellant Percentage
Nitrocellulose 13.10% ± 0.05% Nitrogen 6-12 seconds viscosity	58.50 ± 2.00
Nitroglycerin	15.88 ± 1.00
Diethyleneglycol Dinitrate (DEGDN)	24.82 ± 1.50
Akardit II	0.70 ± 0.20
Magnesium Oxide	0.04 Max
Graphite	0.04 Max
TOTAL:	100.00†

† These values were provided by Radford AAP. The mean values for the constituents do not total 100%.

Appendix B: INDIVIDUAL PLATE SCORES

TOXICITY DETERMINATION WITH TA100

COMPOUND	DOSE/plate	PLATE 1	PLATE 2	PLATE 3
NEGATIVE CONTROL (Start Run)		116	88	102
TP056	5.0 mg	63	72	70
TP056	1.0 mg	61	75	68
TP056	0.2 mg	72	78	80
TP056	0.04 mg	82	95	98
TP056	0.008 mg	85	97	83
TP056	0.0016 mg	86	87	87
NEGATIVE CONTROL (End Run)		101	87	101

Appendix B (cont.): INDIVIDUAL PLATE SCORES

MUTAGENICITY TESTS WITHOUT S-9

COMPOUND	DOSE/plate	TA97	TA98	TA100	TA102
NEG CONTROL		49	25	91	190
(start run)		51	31	113	183
		66	30	108	180
NEG CONTROL		65	21	97	176
(END RUN)		54	32	116	192
		54	24	123	188
NQNO*	2.0 µg			732	731
				615	630
				102	710
TP056	5.0 mg	30	24	106	188
		35	21	108	206
		18	15	94	150
TP056	1.0 mg	72	24	102	232
		55	25	86	212
		76	27	94	178
TP056	0.2 mg	81	30	107	194
		82	30	94	215
		86	19	85	206
TP056	0.04 mg	73	20	98	184
		76	26	115	223
		48	25	118	183
TP056	0.008 mg	52	26	89	168
		56	28	91	168
		44	25	92	200
TP056	0.0016 mg	60	22	84	161
		31	28	109	146
		66	20	89	166

* 4-nitroquinoline-n-oxide

Appendix B (cont.): INDIVIDUAL PLATE SCORES

MUTAGENICITY TESTS WITH S-9

COMPOUND	DOSE/plate	TA97	TA98	TA100	TA102
NEG CONTROL		65	34	89	262
(Start Run)		45	38	83	271
		70	33	90	252
NEG CONTROL		97	26	86	273
(End Run)		85	38	108	266
		80	37	110	246
2-aminofluorene	2.0 µg	327	1347	530	358
		362	1095	557	392
		368	1028	553	412
benzo[a]pyrene	2.0 µg		500	492	
			532	485	
			553	436	
2-aminoanthracene	2.0 µg		1186	1198	
			924	1144	
			1474	1055	
TP056	5.0 mg	18	12	82	250
		12	16	86	152
		18	12	95	164
TP056	1.0 mg	83	22	95	210
		76	27	87	196
		85	20	105	249
TP056	0.2 mg	83	30	91	236
		65	37	89	135
		78	41	80	258
TP056	0.04 mg	87	33	63	249
		82	39	91	254
		63	26	84	248
TP056	0.008 mg	69	22	80	234
		61	30	75	244
		53	29	76	277
TP056	0.0016 mg	59	28	71	248
		62	29	80	218
		58	31	81	248

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